

## PEER REVIEW HISTORY

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### ARTICLE DETAILS

<b>TITLE (PROVISIONAL)</b>	Trends and differences in sexual practices and sexually transmitted infections in men who have sex with men only (MSMO) and men who have sex with men and women (MSMW): a repeated cross-sectional study in Melbourne, Australia
<b>AUTHORS</b>	Martin-Sanchez, Mario; Case, Richard; Fairley, Christopher; Hocking, Jane; Bradshaw, Catriona; Ong, Jason; Chen, Marcus; Chow, Eric

### VERSION 1 – REVIEW

<b>REVIEWER</b>	R. Colby Passaro Department of Emergency Medicine, Los Angeles County + University of Southern California Medical Center, Los Angeles, CA, USA
<b>REVIEW RETURNED</b>	24-Mar-2020

<b>GENERAL COMMENTS</b>	<p>This paper makes an important contribution to the study of the sexual practices and STI prevalence rates among MSM(W) in Australia. It is well organized and easy to interpret. At the same time, I think several adjustments to the language used, a simplification of the statistical approach, and more profound dive into parts of the discussion would enhance the paper. Thank you for the opportunity to review.</p> <p><b>ABSTRACT:</b> P3, L13, L57: Please reconsider use of the stigmatizing language, "bridging population."</p> <p><b>INTRODUCTION:</b> P5, L10: "a 80% increase" should read "an 80% increase" P5, LL 10-17: Please specify whether these rate increases were in a specific population. P5, L26: What does "HIV notification rate" mean? Is this just new HIV cases? P5, L40: Please reconsider use of the stigmatizing language, "bridging population." Why would transmission from bisexual MSM result in a "recent rise?" Is there an increase in MSMW in Australia, or an increase in their interaction with the heterosexual population? Have there been no changes in condom use and safe sex practices look among the heterosexual population?</p> <p><b>METHODS:</b> P6, L47: Why is it reasonably hypothesized that the characteristics of MSMW who predominantly have male partners are similar to MSMO and MSMW who have predominantly female partners are similar to MSWO? Please provide references.</p>
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	<p>P7, L40: Is this approach appropriate - does this not assume some men tested negative for gonorrhoea who were not tested for gonorrhoea at all?</p> <p><b>RESULTS:</b>  P9, LL 10-15: Are there other papers that divide MSMW (MSMW-W and MSMW-M) by these categories and show a significant difference between them? I do not think this distinction adds much to the analysis and am unsure exactly how these two groups are really unique.</p> <p><b>DISCUSSION:</b>  P12, LL47-54: These two sentences are only peripherally related as RAI is not the only risk factor for HIV infection. Please elaborate - for example, the data in P13, LL 24-26 supports the historical difference in HIV infection.  P14, L 6: Please reconsider the stigmatizing language, "bridge."  P14, LL 3-33: Please take another look at this paragraph. There are several grammatical errors and the text itself provides a very superficial overview of how MSMW are a uniquely stigmatized group who are also at-risk for STIs.</p> <p><b>CONCLUSION:</b>  P15, LL10-22: This statement reads as a little bit of a generalization. I recommend changing the language to reflect that this was "among our study population," and maybe just limited to condomless sex (as other "sexual risk practices" were not really explored in this study). Also, instead of stating that the changes sexual practices may have led to increased STIs among the female population, I might clarify that these changes increased risk of transmission of STIs to the female partners of the MSMW.</p>
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<b>REVIEWER</b>	Louis MacGregor University of Bristol, UK
<b>REVIEW RETURNED</b>	21-Apr-2020

<b>GENERAL COMMENTS</b>	<p>This is an excellent piece of work with some very insightful findings. The paper is written very clearly and the results are presented well. I only have a few minor points to address:</p> <ol style="list-style-type: none"> <li>1. This study is likely to suffer biases from the use of self-reported data. Such as social desirability bias or recall bias. While studies of this type are all prone to this sort of bias, it might be useful to mention this and address if any measures were taken to limit this. You do touch on the fact that MSMW may hide their orientation etc but I think slightly expanding on what this might mean for your results would also be interesting.</li> <li>2. Also, a limitation of this methodology is selecting only the first appointment for each unique clinic participant. In a way, this is losing any trends within attendees themselves over-time, and instead picking up on the trends observed in 'new to service' attendees. Although I can see the merit in doing it this way, I think addressing the advantages and disadvantages of selecting appointments in this way is important.</li> <li>3. This work does not mention antimicrobial resistance, which is potentially a key concern given these results. With MSMW possibly allowing drug resistance patterns to move between populations. I believe it would be important to at least briefly</li> </ol>
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	<p>mention this point in the discussion, although a full assessment of this is probably beyond the scope of this work.</p> <p>4. One very small last note: the text on the figures was a little small, I think it would help readability to scale those sizes a bit, or have the legend positioned underneath.</p>
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<b>REVIEWER</b>	Fleur van Aar National Institute for Public Health and the Environment.
<b>REVIEW RETURNED</b>	15-Jun-2020

<b>GENERAL COMMENTS</b>	<p><b>Review - Trends and differences in sexual practices and sexually transmitted infections in men who have sex with men only (MSMO) and men who have sex with men and women (MSMW) in Melbourne, Australia</b></p> <p>In Australia, STI rates are increasing among heterosexuals, but the cause is unknown. Men who have sex with both men and women (MSMW) might act as a potential bridging population for STI transmission from MSM to heterosexuals. The authors collected interesting data on sex practices and condom use by male and female partner among MSM and categorized MSM into MSMO and MSMW (MSMW-W and MSMW-M). Differences in sexual behaviour and STI rates between MSMO and MSMW were explored. The study nicely showed that the increase in syphilis positivity coincided with decreased condom use with female partners among MSMW. This may have led to rises in the incidence of STIs in the female population, the authors conclude.</p> <p>My major comment on this work is that the aim of the study, the methods and conclusion do not fully match. The authors cannot confirm the possibility of STI transmission from MSMW to the heterosexual population based on the results of this study among MSM. This still is a hypothesis. Moreover, the number of syphilis infections among MSMW is very small, which should be taken into account for interpretation. I recommend to focus more on the interesting differences between MSMW-W and MSMW-M in a revision. The conclusion on MSMW being a heterogenous group, the recommendations to explore the needs among MSMW and to develop tailored interventions are very nice and fit the results better.</p> <p>Furthermore, I have quite a few but mostly minor comments on this work:</p> <p><b>General</b></p> <p>I recommend the authors to carefully read the manuscript and look at tables again, as there were some typos and inconsistencies. For example:</p> <ul style="list-style-type: none"> <li>- Page 6, line 44-45, typo: 'for chlamydia' in stead of 'or chlamydia'?</li> <li>- Typo in the footnote: tested in stead of teste. I can't find ** in the table, possibly forgotten to put after 'new HIV diagnosis'.</li> <li>- Table S1: Condom use with <b>casual regular</b> partners in the past 12 months for insertive anal sex and Condom use with <b>casual regular</b> partners in the past 12 months for</li> </ul>
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receptive anal sex. Should 'casual regular' be 'regular male' and 'regular female'?

#### **Abstract**

- Results: the results of the logistic regression analysis are missing, probably to cut words. I suggest to add the most important findings of the logistic regression analysis.
- Conclusion: see major comment. The conclusion of MSMW may act as a transmission bridge does not match with the aim and methods, which is comparing sexual behaviour and STI/HIV trends between MSMO and MSMW. Moreover, the last part of the sentence is already known from previous literature (second sentence of the introduction).

#### **Introduction**

Very nice and well written first paragraph.

- In the second paragraph, the authors could provide more information on the epidemiological and genomics studies that propose that bisexual men might act as a bridging population.
- In addition, the authors did not include references of genomics studies, one of the references is too old to suggest that MSMW could contribute to the current rise in STI, and last, the systematic review is mostly based on Chinese studies which might not be representative for Australia. I recommend to include references of genomics studies and, if possible, to use more recent evidence or studies from countries that are more comparable to Australia.
- Do reference 6-9 support the first sentence of the second paragraph (unknown cause of the rise in gonorrhoea and syphilis among heterosexuals)? The studies that are referred to are all about populations on PrEP and/or MSM.
- After reading the introduction, I was wondering how behavioural trends in the heterosexual population look like in Australia. Could rising STI trends also be explained by increases in risky sexual behaviour among heterosexuals?

#### **Methods**

Most parts of the methods are very well written and clear. The change from culture to NAAT must have had an impact on the number of infections. The authors explored the trends for 2011-2014 and 2015-2018 separately due to this change of diagnostic assays, which is a good thing to do.

- Demographics, behavioural characteristics and outcomes could be described in more detail (e.g. categorization and/or definitions). For example, are all syphilis stages or only infectious stages included? And are syphilis infections of unknown duration included or excluded?
- Changes in STI testing policies are explained. It's not clear to me what the testing policies are for rectal gonorrhoea and rectal chlamydia over the full study period (universal testing?). In addition, the oral ct testing policy before the change to routine testing (no testing at all?), syphilis and HIV testing policies are not explained. I suggest to fully explain the testing policies by STI, all anatomical sites and over time.
- Gonorrhoea positivity is calculated as the number of men tested positive divided by the number of men who were

tested or urethral chlamydia to adjust for bias due to changes in the testing policy. However, asymptomatic infections might have been missed previously. Is it possible to show the trends for symptomatic and asymptomatic infections separately or to provide the number and percentage of asymptomatic urethral infections by year since 2015?

- The logistic regression analysis could be more clearly described.
  1. Why did you perform a logistic regression analysis: which research question does it answer?
  2. What is the dependent variable exactly? It is unclear whether the authors created a new variable (STI/HIV) or assessed each STI separately (and by anatomical location or any location)?
  3. Association between sexual practices and STI/HIV positivity: I did not understand this without seeing the results. I think the independent effect of 'sexual practice' (MSMO versus MSMW) on STI/HIV was explored.
  4. How were missing data handled?
  5. Was data from 2011-2014 included or excluded given the change in diagnostics for chlamydia and gonorrhoea? Did the authors explore the impact of including/excluding 2011-2014 data in the logistic regression analysis?

#### **Results**

1. Did testing increase over time? As trends are explored, it would be nice to see some more basic numbers by year: the number of total consultations, number of consultations among MSMO, MSMW, MSMW-W and MSMW-M.
2. Table 1:
  - Why are the percentages calculated without the category 'no information'? It is more common to calculate and show percentages including 'no info' categories.
  - The variable "casual sexual partner" (No casual partner/ casual male partner/ casual female partner/ casual male and female partner/ no information) is missing in Table 1, which is necessary to understand the numbers by condom use.
  - Did you also collect the number of female partners separately?
3. Table 2: only 89 syphilis infections among MSMW over the full study period. Syphilis positivity increased, but the supplementary table shows that the number of infections by year is low (from 1 in 2011 to 19 in 2018). The low number of infections should be taken into account for interpretation. The population that potentially acts as a bridge population for syphilis is very small.
4. Page 10-11: there was a significant difference between MSMO and MSMW-W and there was no difference between MSMW-W and MSMW-M. Was there also a significant difference between MSMW-M and MSMO?
5. Supplementary tables:
  - The differences between MSM-M and MSM-W are interesting results. The authors could consider to give these result more attention. I suppose that the first sentence of the conclusion 'MSMW is a heterogenous group....male partners' refers to these results.

	<p><b>Discussion/conclusion</b></p> <ol style="list-style-type: none"> <li>1. See major comment on the focus on the hypothesis of STI transmission from MSMW to the heterosexual population. The low number of syphilis infections among MSMW should also be discussed with regard to potential bridge for HIV/STI transmission.</li> <li>2. Most limitations are discussed. Another limitation is that other sexual risk behaviours among MSM such as chemsex and group sex are not collected (or included in the study). I recommend to also add strengths of the study.</li> <li>3. The increase in syphilis positivity coincides with increased proportion of MSMW who report sexual risk behaviour. Are there also other explanations possible? For example, it is sometimes difficult or not possible to determine the stage of syphilis infection. Could changes in determining or reporting syphilis stages play a role?</li> <li>4. Urethral gonorrhoea did not differ between MSMO and MSMW. This is an interesting result. I do not fully understand the explanation given in the discussion "that urethral gonorrhoea is commonly acquired from no anal sexual activity".</li> </ol>
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## VERSION 1 – AUTHOR RESPONSE

### RESPONSE TO REVIEWERS

#### **Reviewer 1:**

This paper makes an important contribution to the study of the sexual practices and STI prevalence rates among MSM(W) in Australia. It is well organized and easy to interpret. At the same time, I think several adjustments to the language used, a simplification of the statistical approach, and more profound dive into parts of the discussion would enhance the paper. Thank you for the opportunity to review.

#### **ABSTRACT:**

P3, L13, L57: Please reconsider use of the stigmatizing language, "bridging population."

Response: Thank you for your comment. We have removed the term 'bridging population' and changed both paragraphs of the abstract according to your comment:

Abstract, Objectives: "In the last decade, there has been an increase in sexually transmitted infections (STI) in men who have sex with men (MSM) in Australia, and since 2015 also in urban heterosexuals. Men who have sex with men and women (MSMW) have characteristics that may differ from both MSM only (MSMO) and heterosexual men. We aimed to compare the sexual practices and the trends in HIV/STI positivity between MSMO and MSMW."

Abstract, Conclusion: "MSMW have overall lower condomless sex and lower HIV/STI positivity. In the last years, changes in the sexual practices in MSM have affected both MSMW and MSMO leading to an increased risk of STI."

#### **INTRODUCTION:**

P5, L10: "a 80% increase" should read "an 80% increase"

Response: Thank you we have corrected the typo.

P5, LL 10-17: Please specify whether these rate increases were in a specific population.

Response: We clarified the population referring to that rate:

Introduction, 1<sup>st</sup> paragraph: "Among the Australian population, there was an 80% increase in the notification rate of gonorrhoea"

P5, L26: What does "HIV notification rate" mean? Is this just new HIV cases?

Response: HIV notification rate refers to the number of new cases reported to the infectious diseases surveillance system during a determined period. It is comparable to new HIV diagnosis, nevertheless there might be some delay in reporting the notification rate from one year to another. We used HIV notification rate to keep the same terminology than the cited document. For gonorrhoea, syphilis and chlamydia the reported rates also correspond to notification rates, we have specified that in the text. Introduction, 1<sup>st</sup> paragraph: "... there was an 80% increase in the notification rate of gonorrhoea (from 65.5 to 118.0 per 100,000), a 14% increase in chlamydia (from 364.5 to 416.8 per 100,000) and more than a two-fold increase in syphilis (from 7.8 to 18.3 per 100,000) between 2013 and 2017.<sup>3</sup>"

P5, L40: Please reconsider use of the stigmatizing language, "bridging population." Why would transmission from bisexual MSM result in a "recent rise?" Is there an increase in MSMW in Australia, or an increase in their interaction with the heterosexual population? Have there been no changes in condom use and safe sex practices look among the heterosexual population?

Response: Thank you for your suggestion. We have removed the term 'bridging population' in the revised version. We hypothesized the recent rise of gonorrhoea and syphilis among heterosexuals could be due to the rise in STI among MSM. That means, if the proportion of bisexual MSM remains constant and the prevalence of STI among MSM is increasing, it could lead to higher odds of females acquiring an STI from bisexual men. We acknowledge this is a hypothesis and do not have any data to support this and therefore we have removed the first sentence in paragraph 2. Similarly, there has been no data examining the interaction between MSM and heterosexual population. Unfortunately, data on sexual practices among heterosexuals are very limited. There were only two population-based sexual health surveys in Australia, one was conducted in 2001-2002 and the second one was conducted in 2012-2013. There has been no trend data on sexual practices among heterosexuals in the late 2010s. We have revised the first few sentences of paragraph 2 accordingly.

Introduction, 2<sup>nd</sup> paragraph: "Past studies have shown that sexual practices among MSM have changed over time (e.g. an increase in condomless sex).<sup>6-9</sup> However, there have been very limited studies examining whether there are any differences in sexual practices between men who have sex with men only (MSMO) and men who have sex with men and women (MSMW or bisexual MSM).<sup>1,10-12</sup>"

#### METHODS:

P6, L47: Why is it reasonably hypothesized that the characteristics of MSMW who predominantly have male partners are similar to MSMO and MSMW who have predominantly female partners are similar to MSWO? Please provide references.

Response: This concept is purely hypothetical, and we could not find any similar studies comparing these populations. We therefore have removed the corresponding sentence in the manuscript. "We hypothesized that the characteristics of MSMW who predominately have male partners are similar to MSMO; while MSMW who predominately have female partners are similar to men who have sex with women only."

P7, L40: Is this approach appropriate - does this not assume some men tested negative for gonorrhea who were not tested for gonorrhea at all?

Response: Thank you for your comment. Urethral gonorrhoea infections in men are usually symptomatic and therefore, we believe this approach is appropriate and it has been used in previous publications. We would like to clarify that we only applied this approach when calculating urethral gonorrhoea. For gonorrhoea at extragenital sites (i.e. anorectal and oropharyngeal), we calculated the prevalence among those who had tested for gonorrhoea only. This approach may not be optimal to estimate the positivity at a certain point of time, but it offers more unbiased and reliable trend estimates.

#### RESULTS:

P9, LL 10-15: Are there other papers that divide MSMW (MSMW-W and MSMW-M) by these

categories and show a significant difference between them? I do not think this distinction adds much to the analysis and am unsure exactly how these two groups are really unique.

Response: To our best knowledge, we could only identify publications comparing MSMO and MSMW. We could not find any publications comparing MSMW-W and MSMW-M and therefore we believe our findings are novel and could fill in the literature gap.

#### DISCUSSION:

P12, LL47-54: These two sentences are only peripherally related as RAI is not the only risk factor for HIV infection. Please elaborate - for example, the data in P13, LL 24-26 supports the historical difference in HIV infection.

Response: Thank you for your comments. We agree RAI is not the only risk factor for HIV infection and therefore we have added the following sentences to the paragraph.

Discussion, 2<sup>nd</sup> paragraph: "Other factors such as drug and alcohol use, other risky practices (e.g. group sex, paying for sex), frequent HIV testing and the use of biomedical interventions (e.g. PrEP and PEP) are also associated with HIV acquisition,<sup>18-20</sup> and very limited studies examining these factors between MSMO and MSMW."

In addition, we have also added a sentence in the limitation paragraph to acknowledge other sexual practices that associated with HIV/STI were not collected in this study.

Discussion, 6<sup>th</sup> paragraph: "Third, other sexual practices that may increase the risk of HIV/STI (e.g., chemsex, group sex, rimming or saliva use as a lubricant)<sup>37-39</sup> were not routinely collected in the clinic and hence not included in the study."

P14, L 6: Please reconsider the stigmatizing language, "bridge." & P14, LL 3-33: Please take another look at this paragraph. There are several grammatical errors and the text itself provides a very superficial overview of how MSMW are a uniquely stigmatized group who are also at-risk for STIs.

Response: We have updated this paragraph of the discussion according to your comments.

Discussion, 5<sup>th</sup> paragraph: "The differential sexual practices and HIV/STI positivity among MSMW<sup>32,33</sup> may also be due to other health issues such as mental health and substance use, that also place them as an especially vulnerable population.<sup>34,35</sup> Bisexual men may face unique stressors, such as specific prejudices against them, pressures or negative attitudes from both heterosexual and LGBT individuals, which can have a negative impact on their health.<sup>35,36</sup>"

#### CONCLUSION:

P15, LL10-22: This statement reads as a little bit of a generalization. I recommend changing the language to reflect that this was "among our study population," and maybe just limited to condomless sex (as other "sexual risk practices" were not really explored in this study). Also, instead of stating that the changes sexual practices may have led to increased STIs among the female population, I might clarify that these changes increased risk of transmission of STIs to the female partners of the MSMW.

Response: Thank you for your suggestion, we revised the conclusion and it reads:

"In our study population, MSMW were a heterogeneous group in which sexual practices and STI positivity varied between MSMW with mainly female partners and MSMW with mainly male partners. Compared to MSMO, MSMW were less likely to engage in condomless sex and had a lower HIV/STI positivity. From 2011 to 2018, changes in the sexual practices in MSM have affected both MSMW and MSMO leading to an increased risk of STI in both subgroups. Further studies also including heterosexual men and women are needed to better understand the recent changes in the STI epidemiology."

#### **Reviewer 2**

This is an excellent piece of work with some very insightful findings. The paper is written very clearly and the results are presented well. I only have a few minor points to address:

Response: Thank you, we appreciate your positive feed-back and the interesting points you raised.



1. This study is likely to suffer biases from the use of self-reported data. Such as social desirability bias or recall bias. While studies of this type are all prone to this sort of bias, it might be useful to mention this and address if any measures were taken to limit this. You do touch on the fact that MSMW may hide their orientation etc but I think slightly expanding on what this might mean for your results would also be interesting.

Response: We have included a phrase in the discussion regarding the possible biases of the use of self-reported data. Besides that, we have expended the section of the discussion regarding the factor that may render MSMW a more hidden population.

Discussion, 6<sup>th</sup> paragraph: "First, data were self-reported by MSHC clients which may influence the results regarding sexual practices, including the number of MSMW and MSM due to social desirability or recall bias. However, the use of a computer-assisted self-interview to collect sexual history at the clinic has shown to improve the accuracy and reduce biased information."

Discussion, 5<sup>th</sup> paragraph: "The differential sexual practices and HIV/STI positivity among MSMW<sup>32,33</sup> may also be due to other health issues such as mental health and substance use, that also place them as an especially vulnerable population.<sup>34,35</sup> Bisexual men may face unique stressors, such as specific prejudices against them, pressures or negative attitudes from both heterosexual and LGBT individuals, which can have a negative impact on their health.<sup>35,36</sup> This factors may also render bisexual men less likely to discuss their sexual health and disclose their sexual orientation with their GPs<sup>11</sup> and, hence not receive the appropriate sexual health care and management."

2. Also, a limitation of this methodology is selecting only the first appointment for each unique clinic participant. In a way, this is losing any trends within attendees themselves over-time, and instead picking up on the trends observed in 'new to service' attendees. Although I can see the merit in doing it this way, I think addressing the advantages and disadvantages of selecting appointments in this way is important.

Response: Thank you, we think this is an important point and we have mentioned that in the methods section.

Methods, 2<sup>nd</sup> paragraph: "Clients who were male, aged 18 years and above, reported having sex with another man in the past 12 months, and attended MSHC for the first time between 2011 and 2018 were included in this analysis. We only include data from the client's first visit to MSHC to avoid any bias including men with repeated visits as they might be at a higher risk of HIV/STI with different sexual practices."

We have also added the following sentence about the disadvantage in the limitation section.

Discussion, 6<sup>th</sup> paragraph: "Fourth, we only included individuals who attended the clinic for the first time. Sexual practices might change with age and further longitudinal cohort studies examining the changes in sexual practices among individuals would be required."

3. This work does not mention antimicrobial resistance, which is potentially a key concern given these results. With MSMW possibly allowing drug resistance patterns to move between populations. I believe it would be important to at least briefly mention this point in the discussion, although a full assessment of this is probably beyond the scope of this work.

Response: Thank you for this suggestion. We have added two sentences addressing the antimicrobial resistance in the Discussion.

Discussion, 3<sup>rd</sup> paragraph: "Furthermore, the rise in antimicrobial resistance in STI,<sup>28</sup> particularly gonorrhoea, is of particular concern. Transmission of antimicrobial resistance across risk populations (e.g. between MSMW and female) may have occurred.<sup>12,29</sup>"

4. One very small last note: the text on the figures was a little small, I think it would help readability to scale those sizes a bit, or have the legend positioned underneath.

Response: Thank you for your comment we have rescaled the figures and adapted the text of the figures.

### **Reviewer 3**

In Australia, STI rates are increasing among heterosexuals, but the cause is unknown. Men who have sex with both men and women (MSMW) might act as a potential bridging population for STI

transmission from MSM to heterosexuals. The authors collected interesting data on sex practices and condom use by male and female partner among MSM and categorized MSM into MSMO and MSMW (MSMW-W and MSMW-M). Differences in sexual behaviour and STI rates between MSMO and MSMW were explored. The study nicely showed that the increase in syphilis positivity coincided with decreased condom use with female partners among MSMW. This may have led to rises in the incidence of STIs in the female population, the authors conclude.

My major comment on this work is that the aim of the study, the methods and conclusion do not fully match. The authors cannot confirm the possibility of STI transmission from MSMW to the heterosexual population based on the results of this study among MSM. This still is a hypothesis. Moreover, the number of syphilis infections among MSMW is very small, which should be taken into account for interpretation. I recommend to focus more on the interesting differences between MSMW-W and MSMW-M in a revision. The conclusion on MSMW being a heterogeneous group, the recommendations to explore the needs among MSMW and to develop tailored interventions are very nice and fit the results better.

Response: Thank you for reviewing our manuscript and for all your relevant comments and suggestions. In this study we wanted to investigate the characteristics of men who have sex with men and women. We agree with the reviewer that this was an observational study and we could not conclude STI transmission from MSMW to the heterosexual population and therefore we have removed the relevant sentences and toned down the message of 'bridging population'.

Furthermore, I have quite a few but mostly minor comments on this work:

#### General

I recommend the authors to carefully read the manuscript and look at tables again, as there were some typos and inconsistencies. For example:

- Page 6, line 44-45, typo: 'for chlamydia' in stead of 'or chlamydia'?

Response: Thank you, we have corrected the typo.

- Typo in the footnote: tested in stead of teste. I can't find \*\* in the table, possibly forgotten to put after 'new HIV diagnosis'.

Response: Thank you, we have corrected the typo in the footnote.

- Table S1: Condom use with casual regular partners in the past 12 months for insertive anal sex and Condom use with casual regular partners in the past 12 months for receptive anal sex. Should 'casual regular' be 'regular male' and 'regular female'?

Response: Thank you for your comment. We have corrected the typo and replaced "casual regular" to "regular male". Both rows refer to condom use with regular male partners one for receptive anal sex and the other for insertive anal sex.

#### Abstract

- Results: the results of the logistic regression analysis are missing, probably to cut words. I suggest to add the most important findings of the logistic regression analysis

Response: We have included the main results from the logistic regression analysis.

Abstract, results: "MSMO had higher odds of testing positive for gonorrhoea (adjusted odd ratio [aOR] 1.36, 95% confidence interval [CI] 1.13 to 1.64), chlamydia (aOR 1.39, 95% CI 1.16 to 1.67), syphilis (aOR 1.74, 95% CI 1.37 to 2.22) and HIV (aOR 4.60, 95% CI 2.43 to 8.70) than MSMW."

- Conclusion: see major comment. The conclusion of MSMW may act as a transmission bridge does not match with the aim and methods, which is comparing sexual behaviour and STI/HIV trends between MSMO and MSMW. Moreover, the last part of the sentence is already known from previous literature (second sentence of the introduction).

Response: We appreciate your comment. We have changed the conclusion of the abstract to:

Abstract, conclusion: "MSMW have overall lower condomless sex and lower HIV/STI positivity. In the last years, changes in the sexual practices in MSM have affected both MSMW and MSMO leading to an increased risk of STI."

#### Introduction

Very nice and well written first paragraph.

- In the second paragraph, the authors could provide more information on the epidemiological and genomics studies that propose that bisexual men might act as a bridging population.

- In addition, the authors did not include references of genomics studies, one of the references is too old to suggest that MSMW could contribute to the current rise in STI, and last, the systematic review is mostly based on Chinese studies which might not be representative for Australia. I recommend to include references of genomics studies and, if possible, to use more recent evidence or studies from countries that are more comparable to Australia.

Response: Thank you for your comment. As per your major comments about the STI transmission between populations, and the comments from other reviewers, we have largely revised paragraph 2 by removing the context of bridging transmission as this is a hypothetical theory. In addition, there has been no Australian epidemiological study examining this bridging pattern and therefore in paragraph 2 we described this as the literature gap and the rationale of this study.

Introduction, 2nd paragraph: "Past studies have shown that sexual practices among MSM have changed over time (e.g. an increase in condomless sex).<sup>6-9</sup> However, there have been very limited studies examining whether there are any differences in sexual practices between men who have sex with men only (MSMO) and men who have sex with men and women (MSMW or bisexual MSM).<sup>10-12</sup> Therefore, this study aimed to compare the demographic characteristics and sexual practices, and describe the trends in HIV/STI positivity in men who have sex with men only (MSMO) and MSMW attending a large sexual health clinic in Melbourne between 2011 and 2018."

As per the suggestion from Reviewer 2, we have added a few sentences on antimicrobial resistance, and we have included two references of genomics studies (Kwong et al. STI. 2018; Williamson et al. Nature Com. 2019).

Discussion, 3<sup>rd</sup> paragraph: "Furthermore, the rise in antimicrobial resistance in STI,<sup>28</sup> particularly gonorrhoea, is of particular concern. Transmission of antimicrobial resistance across risk populations (e.g. between MSMW and female) may have occurred.<sup>12,29</sup>"

Do reference 6-9 support the first sentence of the second paragraph (unknown cause of the rise in gonorrhoea and syphilis among heterosexuals)? The studies that are referred to are all about populations on PrEP and/or MSM.

Response: We have revised the second paragraph and the wrong references have been removed.

After reading the introduction, I was wondering how behavioural trends in the heterosexual population look like in Australia. Could rising STI trends also be explained by increases in risky sexual behaviour among heterosexuals?

Response: That could be the case, but information regarding changes in sexual behaviour among heterosexuals are scarce at a population level. The Australian Study of Health and Relationships surveys reported there was no change in condomless penile-vaginal sex nor in the number of sexual partners among women between 2001 and 2013. Nevertheless, there are no more recent estimates, and hence it is unclear whether this proportion has changed since 2013 along with the increase in STI rates. Given the uncertainty, we reworded the paragraph as follows:

Introduction, 2nd paragraph: "Past studies have shown that sexual practices among MSM have changed over time (e.g. an increase in condomless anal sex).<sup>6-9</sup> However, there have been very limited studies examining whether there are any differences in sexual practices between men who have sex with men only (MSMO) and men who have sex with men and women (MSMW or bisexual MSM).<sup>10-12</sup> Therefore, this study aimed to compare the characteristics and sexual practices, and describe the trends in HIV/STI positivity in MSMO and MSMW attending a large sexual health clinic in Melbourne between 2011 and 2018. Additionally, we aimed to explore differences within MSMW depending on the most frequent gender of their sexual partners."

#### Methods

Most parts of the methods are very well written and clear. The change from culture to NAAT must have had an impact on the number of infections. The authors explored the trends for 2011-2014 and 2015-2018 separately due to this change of diagnostic assays, which is a good thing to do.

Response: Thank you for your positive feedback and recommendations.

- Demographics, behavioural characteristics and outcomes could be described in more detail (e.g. categorization and/or definitions). For example, are all syphilis stages or only infectious stages included? And are syphilis infections of unknown duration included or excluded?

Response: We have expanded the section explaining HIV/STI positivity results. Due to study length constraints, we have not included detailed information on the categorization of demographics and sexual practices. Nevertheless, this information is available in cited manuscript published by the MSHC team.

Methods, 3<sup>rd</sup> paragraph: "We also extracted the HIV/STI testing results on the day. STI testing results included syphilis (serologically confirmed by rapid plasma regain test [RPR], *T. pallidum* enzyme immunoassay [EIA] and *T. pallidum* particle agglutination assay [TPPA], and including all syphilis stages), (...)"

- Changes in STI testing policies are explained. It's not clear to me what the testing policies are for rectal gonorrhoea and rectal chlamydia over the full study period (universal testing?). In addition, the oral ct testing policy before the change to routine testing (no testing at all?), syphilis and HIV testing policies are not explained. I suggest to fully explain the testing policies by STI, all anatomical sites and over time.

Response: Thank you for your comment, we have specified all testing policies during the study period.

Methods, 4<sup>th</sup> paragraph: "In addition, routine screening for oropharyngeal chlamydia among all MSM at MSHC were introduced in April 2017; before then, only MSM who reported as a contact of infection were tested. Routine screening for HIV, syphilis, oropharyngeal and anorectal gonorrhoea, and anorectal chlamydia were conducted among all MSM and the screening guidelines did not change over the study period at MSHC."

- Gonorrhoea positivity is calculated as the number of men tested positive divided by the number of men who were tested or urethral chlamydia to adjust for bias due to changes in the testing policy. However, asymptomatic infections might have been missed previously. Is it possible to show the trends for symptomatic and asymptomatic infections separately or to provide the number and percentage of asymptomatic urethral infections by year since 2015?

Response: Thank you for your suggestion. We do not have a variable to distinguish whether the gonorrhoea infection was symptomatic or asymptomatic. To show the trends for symptomatic and asymptomatic infection, this would require reviewing the medical files for all 470 cases. However, our current ethics does not cover chart review, and this will require additional ethics approval.

- The logistic regression analysis could be more clearly described.

1. Why did you perform a logistic regression analysis: which research question does it answer?

Response: We wanted to assess the independent association between sexual practice (MSMW vs MSMO) and HIV/STI positivity to determine whether MSMW had different odds of having a positive result for syphilis, gonorrhoea, chlamydia and HIV. We reworded the paragraph regarding logistic regression analysis in the methods section accordingly:

Methods, 4<sup>th</sup> paragraph: "Univariable and multivariable logistic regression was used to examine the association between sexual practice (MSMW or MSMO) and HIV/STI positivity. We assessed HIV and each STI separately, and this included (1) gonorrhoea at any anatomical site, (2) chlamydia at any anatomical site, (3) syphilis, (4) new HIV diagnosis on the day of the first visit and (5) previous HIV diagnosis as the dependent variables. Therefore, five different logistic regression models were conducted, and the independent variables included sexual practice (being MSMW or MSMO and using MSMW as reference), the year of the visit and all potential confounding factors (i.e., variables with  $P < 0.20$  in the univariable analyses) in the multivariable analysis. Missing data were presented as 'no information'. We repeated the same procedure using sexual practice categorised in MSMO,

MSMW-M and MSMW-W as the independent variable with MSMW-W as the reference group. We reported the crude odds ratio (OR) and adjusted odds ratio (aOR), and their respective 95% confidence intervals (CI). The 0.05 level was used for statistical significance in all the analysis.”

2. What is the dependent variable exactly? It is unclear whether the authors created a new variable (STI/HIV) or assessed each STI separately (and by anatomical location or any location)?

Response: Thank you for your comment. The dependent variable was HIV/STI positivity, and we assessed HIV and each STI separately. We did not look at STI by anatomical location. We have now clarified this in the Method section.

3. Association between sexual practices and STI/HIV positivity: I did not understand this without seeing the results. I think the independent effect of ‘sexual practice’ (MSMO versus MSMW) on STI/HIV was explored.

Response: Yes, the reviewer was correct. We assessed HIV and each STI separately and the independent variable was sexual practice. We have revised the section accordingly.

4. How were missing data handled?

Response: We have included a sentence referring to missing data: Methods, 5<sup>th</sup> paragraph: “Missing data were presented as ‘no information’”

5. Was data from 2011-2014 included or excluded given the change in diagnostics for chlamydia and gonorrhoea? Did the authors explore the impact of including/excluding 2011-2014 data in the logistic regression analysis?

Response: Results from the whole study period were included in the logistic regression analysis and we have specified this in the table caption. Excluding 2011-2014 data in the logistic regression analysis remains the same conclusion. We did not present this result for the consistency of other results.

## Results

1. Did testing increase over time? As trends are explored, it would be nice to see some more basic numbers by year: the number of total consultations, number of consultations among MSMO, MSMW, MSMW-W and MSMW-M.

Response: We have added a new table (Table 1) which includes the number of total consultations, number of consultations among MSMO, MSMW, MSMW-W and MSMW-M. The number of consultations increased over time but the proportion of MSMO and MSMW remains stable.

2. Table 1:

- Why are the percentages calculated without the category ‘no information’? It is more common to calculate and show percentages including ‘no info’ categories.

Response: We have now calculated and show the percentage for the ‘no information’ categories.

- The variable “casual sexual partner” (No casual partner/ casual male partner/ casual female partner/ casual male and female partner/ no information) is missing in Table 1, which is necessary to understand the numbers by condom use.

Response: We have now included the variable “casual sexual partner” in the table.

- Did you also collect the number of female partners separately?

Response: Yes, we have collected the number of female partners separately. The median number of female partners among MSMW was 2 (IQR 1-5). We have included this statistic in Table 1.

3. Table 2: only 89 syphilis infections among MSMW over the full study period. Syphilis positivity increased, but the supplementary table shows that the number of infections by year is low (from 1 in

2011 to 19 in 2018). The low number of infections should be taken into account for interpretation. The population that potentially acts as a bridge population for syphilis is very small.

Response: Thank you for highlighting this. As per our previous comments, we have now removed the term 'bridging' in the main text. We agree with the reviewer that the number of infections remains low. We have added the number of cases (n) and number of test (N) along with the positivity in the main text to improve the transparency. We have revised the following sentences in the Results and Discussion sections.

Results, 8<sup>th</sup> paragraph: "There was a 41.8% increase in syphilis positivity from 5.5% (43/778) in 2011 to 7.8% (152/1917) in 2018 (Ptrend =0.025) in MSMO (Table S4) and more than a six-fold increase in syphilis positivity from 0.9% (1/112) in 2011 to 6.4% (19/299) in 2018 (Ptrend=0.004) in MSMW, although the number of infections remained low in this group (Table S5). HIV positivity did not change in either group between 2011 and 2018 (Table S4-S5)."

Discussion, 1<sup>st</sup> paragraph: "Specifically, we found that condom use with casual female partners remained low during the study period and that condomless anal sex with casual male partners had increased in MSMW, and these results echo the rise in syphilis positivity over the same period. While some sexual risk practices and STI positivity were generally lower in MSMW than in MSMO, their rising rates could indicate more transmission from MSMW to their female partners than had occurred previously"

4. Page 10-11: there was a significant difference between MSMO and MSMW-W and there was no difference between MSMW-W and MSMW-M. Was there also a significant difference between MSMW-M and MSMO?

Response: There were no significant differences between MSMW-M and MSMO. We have specified this in the results section:

Results, 10<sup>th</sup> paragraph: "...however, there was no significant difference in HIV/STI positivity between MSMW-M and MSMW-W nor MSMW-M and MSMO in the adjusted analysis (Table 5)."

5. Supplementary tables:

- The differences between MSM-M and MSM-W are interesting results. The authors could consider to give these result more attention. I suppose that the first sentence of the conclusion 'MSMW is a heterogenous group....male partners' refers to these results.

Response: Thank you for the comments. We have now moved some supplementary tables to the main text.

Discussion/conclusion

1. See major comment on the focus on the hypothesis of STI transmission from MSMW to the heterosexual population. The low number of syphilis infections among MSMW should also be discussed with regard to potential bridge for HIV/STI transmission.

Response: We have switched the focus of the hypothesis and adapted the discussion following your comments. We have also specified that the number of syphilis infections was low among MSMW.

2. Most limitations are discussed. Another limitation is that other sexual risk behaviours among MSM such as chemsex and group sex are not collected (or included in the study). I recommend to also add strengths of the study.

Response: Thank you for your suggestion. We have included this in the limitations.

Discussion, 6<sup>th</sup> paragraph: "Third, other sexual practices that may increase the risk of STI (e.g., chemsex, group sex, rimming or saliva use as a lubricant)<sup>37-39</sup> were not routinely collected in the clinic and hence not included in the study."

3. The increase in syphilis positivity coincides with increased proportion of MSMW who report sexual risk behaviour. Are there also other explanations possible? For example, it is sometimes difficult or not possible to determine the stage of syphilis infection. Could changes in determining or reporting syphilis stages play a role?

Response: Thank you for your comments. We do not believe staging is a factor. First, the syphilis positivity was based on serology but not staging. Second, all syphilis staging was confirmed and reviewed by an experienced sexual health clinician at our clinic.

4. Urethral gonorrhoea did not differ between MSMO and MSMW. This is an interesting result. I do not fully understand the explanation given in the discussion “that urethral gonorrhoea is commonly acquired from no anal sexual activity”.

Response: In previous studies it has been shown that sexual practices other than anal sex (including oral sex, nudging, dipping and rimming) play a significant role in the transmission of urethral gonorrhoea among MSM. We have specified that in the discussion.

Discussion, 4<sup>th</sup> paragraph: “This may be because some urethral chlamydial infections in MSMW are acquired from women and urethral gonorrhoea is commonly acquired from no anal sexual activity such as oral sex.<sup>30,31</sup>”

#### VERSION 2 – REVIEW

<b>REVIEWER</b>	Louis MacGregor University of Bristol United Kingdom
<b>REVIEW RETURNED</b>	31-Jul-2020
<b>GENERAL COMMENTS</b>	My previous comments have been addressed satisfactorily and I wish you luck with your submission.

#### VERSION 2 – AUTHOR RESPONSE

Reviewer(s)' Comments to Author:

Reviewer: 2

Reviewer Name: Louis MacGregor

Institution and Country:

University of Bristol

United Kingdom

Please state any competing interests or state 'None declared': None

Please leave your comments for the authors below

My previous comments have been addressed satisfactorily and I wish you luck with your submission.

Response:

Thank you for your time and dedication to carefully review our manuscript.